

Mechanisms and performance measures in mastery-based incremental repeated acquisition: behavioral and pharmacological analyses

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Abstract

Rationale Low doses of D-amphetamine may enhance learning, depending on the conditions under which learning is studied.

Objective The objective of this study is to evaluate the sensitivity of procedural variations of an incremental repeated acquisition procedure to very low-dose D-amphetamine administration.

Methods A 60-min session began with a one-link chain (single lever press) that incremented to a maximum of a four-link chain using three levers: left (L), right (R), and back (B). Backward (five rats) and forward (five rats) training procedures were used to build the chain. In pseudo-randomized presentations, a performance session (same chain every session) and a learning session (chain differed from session to session) were imposed. Some learning chains had an embedded repeated response (e.g., LRRB), and others had no such repeat (e.g., LRLB). The product of chain length and number of reinforcers divided by total reinforcers was the primary marker of progress during a session (i.e., progress quotient (PQ)). After behavior stabilized, D-amphetamine (0.01–3.0 mg/kg, i.p.) was administered.

Results Acquisition was superior for the backward training group during non-repeating learning sessions, across all but the highest doses of D-amphetamine. Very low, clinically relevant, doses of D-amphetamine improved acquisition for the backward training group during repeating learning sessions.

Conclusions Under some conditions, low doses of D-amphetamine enhanced learning for one training procedure

group. A novel dependent measure (“PQ”) was a superior marker of progress on this mastery-based learning task.

Keywords Backward chaining · D-Amphetamine · Forward chaining · Incremental repeated acquisition · Learning · Lever press · Low dose · Rats

Introduction

The repeated acquisition of behavioral chains (RA) is a procedure that was pioneered by Boren (1963) and Boren and Devine (1968) to study learning (i.e., behavior in transition) and complex behavior using a within-subject experimental design. Moerschbaeche et al. (1979) and Moerschbaeche and Thompson (1980) and Thompson (1973), Thompson and Moerschbaeche (1978, 1980), and Thompson et al. (1983) exploited this procedure to study drug effects on learning. The RA procedure was modified by Pieper (1976) and Weinberger and Killam (1978) to increment to progressively longer chains within a session. This incremental repeated acquisition (IRA) procedure is used to study learning with the added benefit of manipulating task difficulty dynamically.

Repeated acquisition procedures (RA and IRA) typically comprise two components, one that requires subjects to learn a different response sequence each experimental session (the learning component), and one that requires the performance of a response sequence already learned (the performance component). In studies of drug action, the performance component serves as a control for nonspecific drug effects or effects on response rate or psychomotor function. IRA procedures have the additional requirement that pre-set criteria be met on shorter response chains before a longer response chain is introduced; in this way, performance and acquisition on progressively more difficult

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chains can be measured within a single session. In various forms, IRA procedures have been effective in studying neurotoxicant and drug effects on the acquisition of new behavior in individual subjects, showing that learning is differentially affected by drugs from different pharmacological classes (e.g., Cohn and Paule 1995; Paule and McMillan 1984; Wright and Paule 2007).

Different approaches can be taken to incrementing and defining the chain used in the IRA procedure, but the influence of these different approaches on the behavior that occurs is poorly understood. Chain type (defined as the particular structure of sequences that make up a response chain) and training procedure (defined as how chains are lengthened) are both likely to influence the speed and accuracy of acquisition. Wright and Paule (2007) manipulated chain type by varying the number of levers used and the number of presses on adjacent levers. They found that accuracy was inversely related to the number of levers available and directly related to the adjacency of successive levers. Cohn et al. (1993) also reported an effect of chain type such that accuracy was better on chains that resembled the performance chain. In the present study, a different structural feature was examined by comparing chains with and without consecutive repeats. This was done not only because it has been underexplored in the literature but also because low doses of D-amphetamine may produce perseveration (or the persistent repetition of a response). Therefore, some chains were structured such that a tendency to perseverate might be beneficial under some conditions (i.e., backward-trained repeating chains).

With respect to training procedures, “lab lore” (and clinical lore) holds that backward chaining is preferable to forward chaining (Lattal and Crawford-Godbey 1985; Pear 2001; Smith 1999; Weiss 1978), but few studies have examined this issue systematically. The training procedure is a crucial component of IRA, as it determines how the chain will progress in length throughout the session.

Backward chaining (in which each link in the chain is added in front of the current link) is the most widely used approach to training IRA (see Table 1). Forward chaining (in which each link is added behind the current link) would be the alternative. In the present study, training procedure was explicitly manipulated so that both backward and forward chaining could be assessed.

These procedural variations are important for understanding drug effects on behavior. A wide variety of drugs, including the amphetamines, has been used with IRA procedures to assess their effects on learning. D-Amphetamine promotes the activity of dopamine neurotransmitter systems by increasing the release of vesicular dopamine and blocking its re-uptake into the presynaptic neuron. Further, dopamine has been shown to be a key player in reinforcement processes (McGregor and Roberts 1994; Spanagel and Weiss 1999; Wise 2004). Laboratory investigations commonly use doses in the range of 0.1 to 3 mg/kg of D-amphetamine, but this range may be high as compared with doses that are used clinically (Grilly and Loveland 2001), and even lower doses may be necessary.

Some laboratory studies using pigeons or rats have demonstrated selected performance enhancements under RA and IRA procedures at “low” doses of D-amphetamine. A small decrease in errors was reported at 0.3 mg/kg with an RA procedure (Harting and McMillan 1976) and at 0.1 mg/kg with an IRA procedure (Paule and McMillan 1984) during some test conditions. Others have reported no benefit of D-amphetamine in the range of 0.1 to 0.56 mg/kg on an RA procedure in pigeons (Thompson 1973; Thompson et al. 1983; Thompson and Moerschbaecher 1978, 1980; Moerschbaecher and Thompson 1980; Moerschbaecher et al. 1979) or on an IRA procedure in rats (Mayorga et al. 2000). Procedural differences across these studies could account for some of these discrepancies.

The present study was designed to examine two procedural manipulations that have received relatively little

Table 1 How the performance sequences are trained using backward (top panel) and forward chaining (bottom panel)

Backward chaining: R–L–R–B						
Link 4	Link 3	Link 2	Link 1			
			High tone: B	→	Sucrose	
		High pulsing: R	→	High tone: B	→	Sucrose
	Low tone: L	→	High pulsing: R	→	High tone: B	→
Low pulsing: R	→	Low tone: L	→	High pulsing: R	→	High tone: B
						Sucrose
Forward chaining: L–R–B–R						
Link 1	Link 2	Link 3	Link 4			
Low pulsing: L	→	Sucrose				
Low pulsing: L	→	Low tone: R	→	Sucrose		
Low pulsing: L	→	Low tone: R	→	High pulsing: B	→	Sucrose
Low pulsing: L	→	Low tone: R	→	High pulsing: B	→	High tone: R
						Sucrose

attention, namely, the structure of the chain and the type of training procedure used to increase chain length. Additionally, to address the potential benefits of low-dose amphetamine, a range of doses, beginning an order of magnitude lower than many studies, was used. In this way, the sensitivity to low-dose D-amphetamine of two procedural manipulations was assessed. A novel dependent measure that may be better suited for analyzing mastery-based IRA data than other traditional dependent measures was also explored. This measure, called the progress quotient (PQ), combines chain length and reinforcer count into a single overall marker of progress in producing long chains. This measure is defined, and the rationale for its use is described below.

Methods

Subjects

Ten adult male Long Evans rats, housed individually in a temperature- and humidity-controlled AAALAC-accredited colony room, were maintained on a 12-h light–dark cycle (lights on at 7:00 a.m.) with free access to water in their home cages. Weight was maintained at 300 g (± 5 g) by individualized feeding of a Purina® chow diet. A portion of the animal's caloric intake was available during experimental sessions, via 45 mg Purina® sucrose pellets. Public Health Service guidelines for laboratory animal care were followed, and the study was approved by the Auburn University Animal Care and Use Committee.

Apparatus

Ten commercial operant chambers (Med Associates Inc. St. Albans, VT, USA, model #Med ENV 007) containing a lever on the back wall (designated the B lever) and a lever to the left (L lever) and right (R lever) of the pellet dispenser were used. Each chamber measured 12"L \times 9.5" W \times 11.5"H. Pellet dispensers were located between the two front levers and delivered 45 mg sucrose pellets (Purina Mills, Inc., St. Louis, MO, USA). Each chamber was equipped with Sonalert tones™ (2,900 and 4,500 Hz, nominally) calibrated to an amplitude of 70 dBc for presentation of auditory stimuli. A 28-V house light was located at the top of the back wall and illuminated each chamber. Each chamber was surrounded by a sound-attenuating cabinet, each with a built-in ventilating fan which circulated air throughout the experimental chamber and provided white noise for the duration of the experimental session. Programs for experimental procedures and data collection were written using MED-PC IV (Med Associates, St. Albans, VT, USA), and all session events were recorded

with 0.01" resolution. All programming equipment was located in a room adjacent to the testing room.

Procedure

All experimental sessions were conducted at approximately the same time each day, in the same testing room, and sessions ended after 50 reinforcers in the four-link chain were obtained or 1 h had passed. Rats were randomly assigned to either the forward training ($n=5$) or backward training ($n=5$) group. For the forward training group, to build a chain, links were added after each previously learned link. For example, the chain LRBR was trained as follows: L \rightarrow sucrose, L–R \rightarrow sucrose, L–R–B \rightarrow sucrose, and L–R–B–R \rightarrow sucrose. For the backward training group, chains were built by adding a new link before the previously learned link(s). For example, LRBR was trained as follows: R \rightarrow sucrose, B–R \rightarrow sucrose, R–B–R \rightarrow sucrose, L–R–B–R \rightarrow sucrose.

A one-link chain (single response) began each session. Chain length was increased using a pre-set mastery-based criterion. After ten consecutive correct responses (no errors), a second link was added, forming a two-link chain. After five consecutive reinforcers (i.e., correct executions of the two-link chain) a third link was added, and after five consecutive reinforcers, a fourth link was added. Pressing an incorrect lever at any point in the chain resulted in a 2" blackout period in which the house light was darkened and no response was reinforced. Following a blackout period, the current chain length reset to the beginning. Each link in the chain was paired with a discrete auditory stimulus (a low tone, low pulsing tone, high tone, or high pulsing tone, see Table 1).

Animals were exposed first to the performance condition in which they were required to perform the same four-link sequence each session (LRBR for the forward group and RLRB for the backward group). Initially, animals were trained on a three-link chain, but it quickly became apparent that a four-link chain was required to avoid ceiling effects. The fourth link in the performance chains appeared at the end of the chain for the forward group and at the front for the backward group so that both were added to the last place in the chain.

Learning sequences (in which the animals were required to acquire a different response sequence within the experimental session) were introduced only after an animal achieved at least 80% accuracy on the four-link performance chain for three successive days. This stability criterion was reached within 20 sessions for all animals. Two types of learning chains were presented: non-repeating (traditional) and repeating chains. Repeating chains were four-link sequences that included two consecutive responses on the same lever (e.g., LLRB, BRRL, and RLBB). Non-repeating chains were four-link sequences that did not have consecutive responses on the same lever (e.g.,

LRBR, RBLR, and BLBR). All animals received equal, pseudo-randomized presentations of each learning chain type (repeating and non-repeating) and the performance chain. No discriminative stimuli were used to signal which chain type was in effect during a session. Due to constraints on the number of four-response sequences possible across three levers, learning chains were reused. All possible learning chains were used before being reused, and no learning chain was used more than twice.

Drug administration

Drug administration commenced after behavior in both performance and learning conditions showed no systematic trend in overall response rate or PQ for ten consecutive sessions. All animals received D-amphetamine sulfate (Sigma-Aldrich, St. Louis, MO, USA), dissolved in a 0.9% saline solution such that an injection volume of 0.3 ml (0.1 ml/kg) was maintained. The doses ranged from 0.01 to 3.0 mg/kg D-amphetamine, calculated as the salt. All doses of D-amphetamine and saline vehicle were administered intraperitoneally 10 min prior to each experimental session in a room adjacent to the experimental run room.

Drugs were always administered on Tuesdays and Fridays. Saline vehicle was administered on Thursdays and Sundays while Mondays and Wednesdays served as noninjected control sessions. Sessions were conducted such that one performance, learning-repeating and learning-non-repeating session, was conducted at each dose.

Statistical and data analysis

Three dependent measures were analyzed in the present study: total responding (number of responses per session), accuracy (corrects divided by total responses), and a PQ:

$$PQ = (1 \times Rf_1 + 2 \times Rf_2 + 3 \times Rf_3 + 4 \times Rf_4) / Rf_{tot} \quad (1)$$

where Rf_i = number of reinforcers earned on a chain length of length i , and Rf_{tot} = total reinforcers earned in the session. This index serves as a measure of progress and avoids many problems that are associated with using a measure of accuracy or maximum sequence length reached during a mastery-based criterion IRA procedure, such as the one used here. PQ weighs each reinforcer earned by the chain length during which it was earned. Therefore, a reinforcer earned in a four-link chain is favored over one earned in a one-link chain. Maximum chain length reached is also reported for the baseline data.

Interactions between amphetamine and training procedure within each chain type were examined using a two-

way repeated measures analysis of variance (RMANOVA), with dose as a within-subject variable and training procedure (backward or forward chaining) as a between-subjects factor. This was conducted separately for each chain type: performance, learning-repeating, and learning-non-repeating chain types. Interactions between chain type and D-amphetamine within each training procedure were examined in separate analyses using a two-way RMANOVA, with dose and chain type as within-subject variables. This was conducted separately for each training procedure: backward and forward training.

All animals are included in each analysis. The Greenhouse–Geisser correction was used when necessary (i.e., GG epsilon < 0.6). Planned pairwise comparisons between saline and an active dose were conducted if there was a statistical main effect of dose. To correct for multiple comparisons and retain a p value of .05, this value was divided by 7 (the number of comparisons for any dose-effect relationship), so planned comparisons required a p value of .007 to be significant. Post hoc tests conducted on baseline data were corrected for multiple comparisons using the Bonferroni correction. Conclusions about group differences were addressed by examining graphs. All error bars represent the SEM, and $p < .05$ was the criterion for statistical significance. Statistical analyses were conducted using SYStat (San Jose, CA, USA).

Results

Baseline: responding, PQ, accuracy, and maximum chain length

There was no main effect of training procedure (backward vs forward) on any of the dependent measures (all p 's > .18) during baseline (Fig. 1). All animals achieved a four-link chain during performance and learning conditions for baseline sessions although, sometimes, only very few reinforcers were obtained on this long chain during learning conditions (not illustrated). There was a main effect of chain type (performance, repeating-learning, and non-repeating-learning) on PQ ($F(2,16) = 10.039$, $p < .001$) and accuracy ($F(2,16) = 26.574$, $p < .001$) but not on total responding ($p = .165$). No significant interactions were found.

Post hoc analyses were used to clarify the main effect of chain type. This revealed that PQ and accuracy were higher during the performance chain than they were during repeating or non-repeating learning chains (all p 's < .02), although no significant differences between repeating and non-repeating learning chains were detected during baseline.

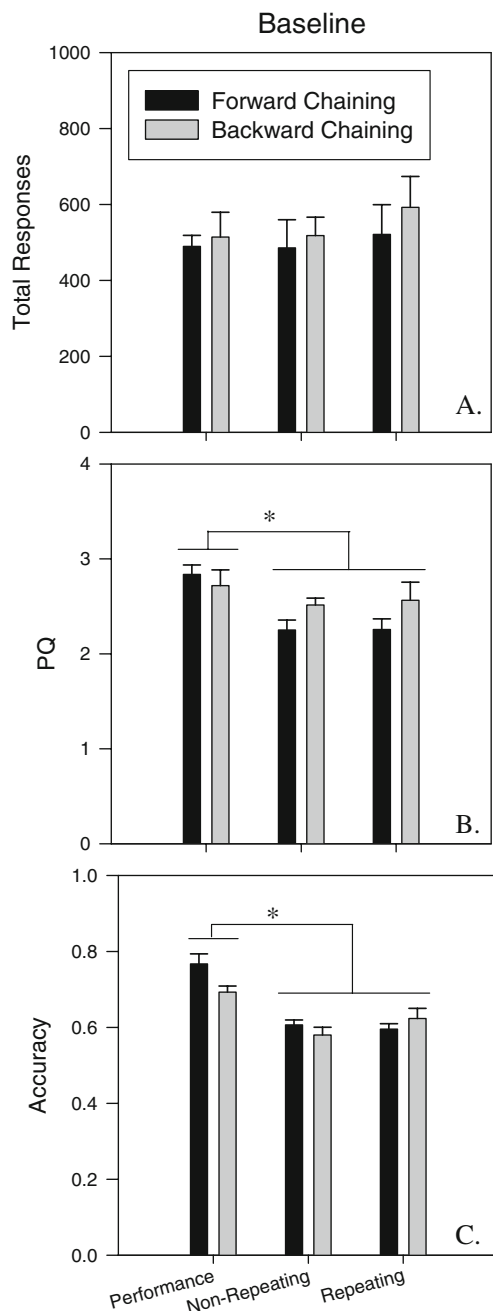


Fig. 1 Baseline responses per session (a), progress quotient scores (b), and accuracy (c) grouped by chain type for each training procedure group are shown. Error bars reflect the SEM. The asterisks indicate significant differences between the learning and performance chain types

Drug phase: total responding

Dose

A main effect of dose was seen for the backward-trained non-repeating ($F(8,32)=10.20$, $p<.001$) and performance ($F(8,32)=3.03$, $p=.012$) chains as well as for the forward

trained non-repeating ($F(8,32)=14.28$, $p<.001$) and performance ($F(8,32)=2.59$, $p=.027$) chains (Fig. 2b). Responding during neither backward ($p=.575$) nor forward ($p=.203$) trained repeating chains was affected by dose (Fig. 2c).

Training procedure

During drug administration, overall response rate was not affected by training procedure for any chain type (Fig. 2a–c, all p 's $>.1$).

Chain type

Within the forward training group, an interaction between chain type and dose occurred for the non-repeating and repeating chains ($F(8,32)=5.05$, $p<.001$) and non-repeating and performance chains ($F(8,32)=5.39$, $p<.001$).

Similarly, within the backward training group, an interaction between chain type and dose occurred for the non-repeating and repeating chains ($F(8,32)=7.15$, $p<.001$) and non-repeating and performance chains ($F(8,32)=6.34$, $p<.001$).

The main effects of dose (and significant interactions) are clarified by post hoc tests, which revealed that responding increased at 1.0 mg/kg for the performance and non-repeating learning conditions (Fig. 2a, b) for the backward training group.

Drug phase: progress quotient (PQ)

Dose

For all conditions, there was a main effect of dose such that low to moderate doses had no effect on PQ or increased it, and higher doses always decreased it. The p values for the main effect of dose were all less than .01 (Fig. 3a–c).

Training procedure

For the performance condition, there was an interaction between dose and training procedure ($F(8,64)=3.280$, $p=.053$). For the non-repeating chains, there was a main effect of training procedure ($F(1,8)=8.67$, $p=.019$), but no interaction with dose. For the repeating chains, there was not a significant interaction between dose and training procedure ($p=.44$; see Fig. 3).

Chain type

For the forward training group, the PQ was generally lower for non-repeating ($F(1,4)=214.86$, $p<.001$) and repeating ($F(1,4)=23.17$, $p=.009$) learning chains, than for the performance chain. Also, scores for the non-repeating

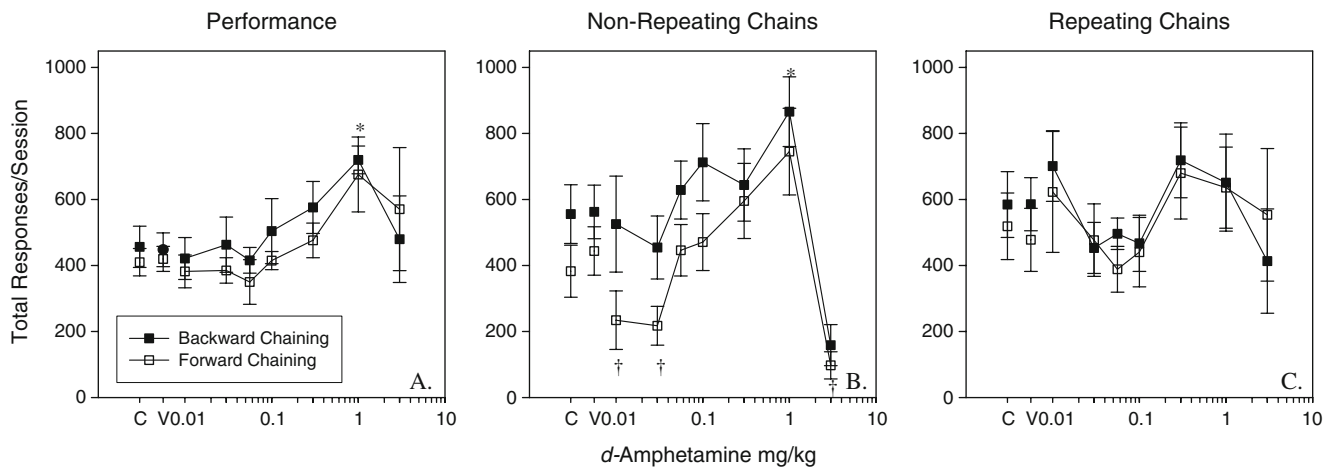


Fig. 2 Responses per session for each training procedure group are plotted for each of the three chain types. Error bars reflect SEM. There are no significant main effects of training procedure ($p < 0.05$) for any chain type. An asterisk and a cross denote significant difference from

vehicle for the backward- and forward-chaining group, respectively ($p \leq 0.007$ for both groups). Control (C), vehicle (V), and D-amphetamine sessions are shown

chains were lower than those for repeating ($F(1,4) = 12.23$, $p = .025$) chains. An interaction between chain type and dose was seen between the forward trained non-repeating and performance chains ($F(8,32) = 4.63$, $p = .034$).

Within the backward training group, non-repeating chains resulted in lower PQ scores than the performance ($F(1,4) = 53.96$, $p = .002$) and repeating ($F(1,4) = 73.95$, $p = .001$) chain types. However, repeating and performance chain types were statistically indistinguishable from each other ($p = .844$). An interaction between chain type and dose was seen between the backward-trained non-repeating and performance chains ($F(8,32) = 4.71$, $p = .047$) and non-

repeating and repeating ($F(8,32) = 4.41$, $p = .044$) chain types.

Post hoc tests and an examination of Fig. 3 helped to clarify these effects which revealed that low doses (0.01–0.1 mg/kg) of D-amphetamine improved PQ when chains containing a repeat were established using a backward training procedure. For other conditions, low to moderate doses were ineffective. At the highest dose, all learning chains were disrupted by D-amphetamine but, for the performance chain, the backward-chaining procedure showed greater disruption than forward chaining, see asterisks on Fig. 3c.

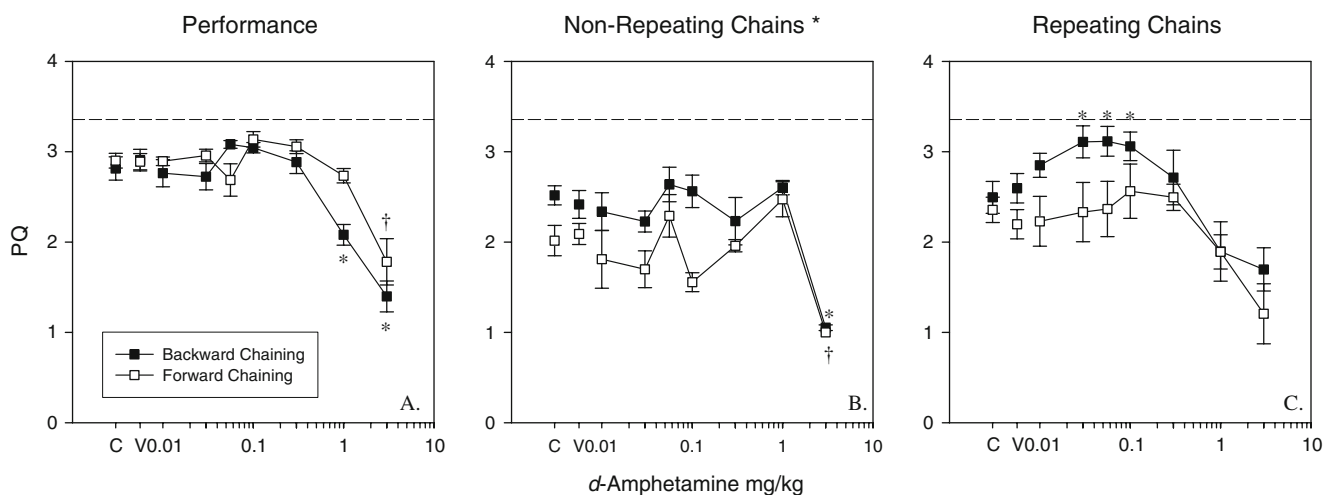


Fig. 3 Progress quotient (PQ) scores for each training procedure group are plotted for each of the three chain types structured similarly as in Fig. 2. An asterisk and a cross denote significant difference from vehicle for the backward- and forward-chaining group, respectively

($p \leq 0.007$ for both groups). The asterisk in the panel title (i.e., non-repeating) denotes a significant main effect of training procedure for that chain type. The dashed line denotes the maximum possible PQ score, 3.357

Drug phase: accuracy

Dose

For all conditions, there was a main effect of dose such that higher doses decreased accuracy. The p values for a main effect of dose were all less than .02.

Training procedure

There was a main effect of training procedure during the performance condition such that forward chaining resulted in higher accuracy than backward chaining ($F(1,8)=7.928$, $p=.023$). No effect of training procedure was evident during either learning chain type (repeating or non-repeating), and the interaction between training procedure and dose did not meet the traditional cutoff p value ($p=.068$).

Chain type

Within the forward training group, accuracy was generally lower for both non-repeating ($F(1,4)=82.700$, $p<.001$) and repeating learning chains ($F(1,4)=33.766$, $p=.004$), than for the performance chain. No significant differences in accuracy appeared between the two learning chains for the forward training group.

Within the backward training group, accuracy for the non-repeating chains was significantly lower than performance ($F(1,4)=27.365$, $p=.006$), this analysis also revealed an interaction between dose and chain type ($F(8,32)=3.921$, $p=.003$).

Post hoc tests did not reveal any systematic improvement in accuracy associated with D-amphetamine for any training procedure/chain type combination. In fact, after correcting for multiple comparisons, the only effects of dose were the decreases in accuracy at the highest doses and occurred during the performance chain only.

Discussion

The current study was designed to examine how two procedural manipulations of an IRA procedure (training procedure and chain type) influence performance and acquisition of response sequences during low-dose D-amphetamine administration. An index, PQ, was developed that measures progress on the mastery-based IRA procedure. PQ along with total responding served as the primary dependent measures for assessing performance and acquisition. The more traditional measure of accuracy (and baseline maximum chain length) was also reported and compared with PQ. The results indicate (1) the effects of training procedure on learning chains depended on chain type, (2) low doses of D-

amphetamine improved PQ for one training procedure/chain type combination, (3) differences in PQ were seen at doses that did not affect overall responding, and (4) PQ is a better measure than accuracy or maximum chain length for mastery-based training procedures.

The conditions used in the present study resulted in the reliable production of four-link chains in rats during control conditions, which suggests that longer chains could have been achieved had the program allowed it. This is longer than those reported by other investigators (using rats), where three-link chains are more typical (Popke et al. 2000a, b; Wright and Paule 2007; Paule and McMillan 1984). It is not clear which aspects of the current procedure are responsible for the longer chains reached, but several procedural considerations can be noted. These include (1) mastery criterion of ten consecutive correct responses for adding the second link and five consecutive correct chains for adding subsequent links, (2) unique auditory discriminative stimuli for each chain link, (3) performance and learning conditions in separate sessions, (4) 1-h sessions, and (5) training the performance chain before introducing learning chains.

PQ, a summary measure of progress

The procedure used in the present study imposed a behaviorally based mastery criterion in which a new link was added only after a shorter length chain was executed reliably and with no errors. This approach helped identify peak performance in individual animals while titrating task demand according to individual performance, but it presents a challenge to the goal of finding a quantitative marker of progress. Two traditional measures, accuracy and maximum chain length, are ill-suited to such a mastery-based approach for the reasons described below.

A single overall measure of accuracy is meaningful only if “correct” and “incorrect” responses are recorded under similar conditions. Here, however, some animals performed many short chains with relatively high accuracy, and others produced much longer chains but sometimes with similar or even lower overall accuracy. A single measure like accuracy does not capture the superior performance of those animals that reached longer chains. Similar difficulties with accuracy arise if the number of responses or reinforcers at each chain length is free to vary. Maximum chain length might also be reported, but this does not distinguish between an animal that met criterion for a four-link chain but never successfully produced such a chain from an animal that received 50 reinforcers (the maximum possible) during the four-link chain. These difficulties might be overcome if accuracy during each chain length is plotted individually and adjusted for maximum chain length attained. However, with multiple dependent and independent variables, this approach is overly cumbersome.

Additionally, accuracy can be heightened (or lowered) artifactually depending on the type of training procedure used to increment the chain. When a forward training procedure is used, the subject can accumulate correct responses more readily than under a backward training procedure. For example, when first responding on a four-link chain (immediately after meeting the criterion on a three-link chain, say), the forward-chaining animal can accumulate correct responses on links 1–3 and only make errors on the fourth link. This cannot occur with backward chaining because the animal begins the response sequence with the novel link and thus if it continues to make errors on the fourth link, it will only accumulate errors. This artifactually increases accuracy on forward-chaining procedures while decreasing it on backward-chaining procedures. Further, this effect explains why there is no significant difference of training procedure on accuracy during the learning chains (Fig. 4b–c). Taken together, these problems with accuracy can explain the differences between accuracy and PQ; because of these, PQ will be the primary dependent measure analyzed here.

PQ is not confounded by chain length reached or the characteristics of the training procedure. PQ, instead, couples the length of the chain reached with the number of reinforcers earned during that chain length. Equation 1 weighs a reinforcer for a four-link chain four times more than a reinforcer for a one-link chain. If the four-link chain was never attained, then the weighting is zero for the four-link chain, so only short-chain reinforcers are counted. Thus, the PQ counts only responses that are part of correctly completed chains and emphasizes long chains. Note that if only four-link chains were included in a session, then the maximum PQ possible would be four. Similarly, if only three-link chains were included, then the maximum PQ would be three. With the mastery criteria imposed, the inclusion of all chain lengths, and the cap on

50 reinforcers for a four-link chain, the maximum PQ possible for the present study was 3.357. One potential weakness with PQ is that it is not directly sensitive to the number of errors made. Errors are influential indirectly, however, by making it difficult to meet the mastery criterion, by contributing to the overall response rate, and by making it difficult to produce a large number of long chains during a time-limited session.

Training procedures and chain structure

The near-ubiquitous use of backward training for the IRA procedure (e.g., Mayorga et al. 2000; Popke et al. 2000a, b; Wenger et al. 2004; Weinberger and Killam 1978) reflects the overall preference for backward training seen in the animal literature. This may be traceable to suggestions that such an approach results in higher accuracy because (1) it strengthens the response closest to primary reinforcement first and works back from there, and (2) each link in the chain will act as a conditioned reinforcer for the next link in the chain (Millenson 1967; Pear 2001, respectively). In this way, as an animal moves through the chain, it moves along a progressively strengthening reinforcement gradient and through stronger conditioned reinforcers. However, it is difficult to locate studies that directly compare forward and backward training with animals. Studies of humans have yielded mixed results, but sometimes have suggested that forward training is superior (Smith 1999; Weiss 1978). Similarly, chain type is rarely manipulated in studies using RA or IRA procedures and is often explicitly held constant so that all chains are roughly equivalent. This approach began with the development of RA procedures (Boren and Devine 1968) and has persisted in the IRA literature. Those who have explicitly manipulated chain type have found it to effect acquisition (Wright and Paule 2007). In the present

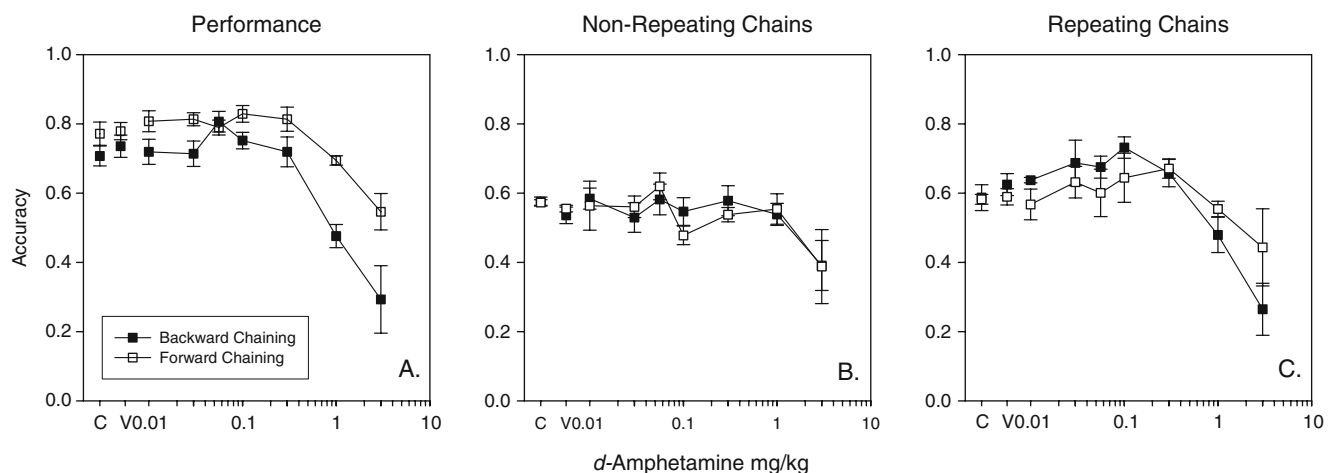


Fig. 4 Accuracy scores for each training procedure group are plotted for each of the three chain types structured similarly as in Figs. 2 and 3. No significant effect of training procedure is detectable in either learning chain type. Decreases in accuracy as a function of dose are seen

study, both training procedure and chain structure were important determinants of PQ, as was experience with the chain. The effects of training procedure and chain structure were amplified as a result of D-amphetamine administration.

D-Amphetamine

Responding and PQ For the performance condition, acute doses of D-amphetamine produced the biphasic dose-effect relationship on response rate commonly seen with psychomotor stimulants. Rate increases appeared at 1.0 mg/kg, and rates decreased from this peak at 3.0 mg/kg. Higher doses, which certainly would have substantially reduced responding, were not examined. The pattern was less clear for the two learning conditions, but a tendency towards a rate increase was apparent at about 0.3–1.0 mg/kg. It is not clear why rate changes were observed at 0.01–0.03 mg/kg of D-amphetamine for one condition, as seen in Fig. 2b. These sessions were examined in detail, and no unusual conditions were evident, and no aberrant responders were noted.

Measures of PQ showed a different pattern of effects. As with overall response rate, the highest dose of D-amphetamine decreased PQ. The doses that had no effect on response rate, however, either systematically increased (backward chaining, repeating chains) or had no overall effect (performance and non-repeating chains) on PQ. For the performance condition, training procedure did not influence D-amphetamine's effect on responding and had no detectable influence on PQ as a function of dose, although visually, it appeared that forward training was slightly more resistant to the detrimental effects of high doses of D-amphetamine than was backward training. For the non-repeating chains, backward training produced higher PQ across all but the two highest doses of D-amphetamine. For the repeating chains (in the learning condition), low doses (0.01–0.1 mg/kg) of D-amphetamine increased PQ when trained using backward chaining. In fact, D-amphetamine increased PQ to levels seen for the performance chain at those doses and to levels that were very close to the maximum possible score. Such improvement did not occur for the forward training group on repeating chains. This indicates that these very low doses can, under some conditions, improve learning.

It is important to note that these doses are much lower than what is typical in the IRA literature and well below doses that altered response rates, but are a much closer approximation of clinical dosing (Grilly and Loveland 2001). This may correspond to the benefits of low-dose amphetamine seen in ADHD populations (Berman et al. 1999; Vyse and Rapport 1989; Harris et al. 1978). Also, these results indicate that the IRA procedure is sensitive enough to detect changes in PQ score at very low doses, even with no detectable change in overall responding.

Performance enhancement by low-dose amphetamine has been reported using baselines designed to examine attention, learning, and memory. Specifically, D-amphetamine increased percent correct responses in some sustained attention tasks (Grilly and Gowans 1988; Grilly and Simon 1994). It facilitated learning and memory in active (Banerjee 1971; Satinder 1971) and passive (Kovacs and de Wied 1978) avoidance tasks, and in radial arm (Dow-Edwards et al. 2008) and water mazes (Brown et al. 2000; Packard and McGaugh 1994). It decreased response (Cole and Robbins 1987) and choice (Grilly et al. 1989) latencies, and in some studies of delayed discounting, amphetamine increased preference for the large, delayed reinforcers (Pitts and McKinney 2005). These effects typically occurred at the lower doses (~0.1–1.0 mg/kg) employed in those studies, while the higher doses decreased responding and increased errors. The IRA procedure draws from several functional domains, including attention, reinforcer impact, and memory. It is possible that the amphetamines' enhancement of these domains contributed to the improved performance on the IRA here.

One other possible explanation for the enhanced learning of backward, repeating chains can be offered. D-Amphetamine induces perseveration on IRA (Paule and McMillan 1984) and other behavioral tasks (Spanagel and Weiss 1999; Wise 2004) such that repeating a response is made more likely while under the influence of this drug. This is exactly what is required in the repeating chains. The way that backward training builds a chain may also be relevant since it allows the animal to execute responses that have recently been reinforced as it moves through the chain.

Accuracy When accuracy was examined, no differences in behavior between the forward- and backward training groups, during either of the learning chain types, were detected. Also, no effect of low-dose D-amphetamine was evident for any chain type/training procedure combination. What is apparent, however, is an effect of training procedure during the performance component such that the forward training group had slightly higher accuracy than the backward training group, as a function of dose. The divergence in the data between accuracy and PQ can be explained by the problems discussed above, and for those reasons, the effects detected on accuracy are considered generally unreliable, and are presented so that a comparison between PQ and a traditional dependent measure can be made.

Concluding remarks

The IRA procedure used here had several characteristics (described above) that appear to have resulted in a high level of performance and learning of four-response sequences.

Chain lengths of four were readily acquired within 20 sessions with such high accuracy that ceiling effects were noted when accuracy was examined as a function of chain length. A similar finding has been seen with BALB/c mice in our laboratory (unpublished data). The success of this IRA procedure, coupled with its ability to provide multiple chain lengths within a session, suggests that the approach used here could prove useful when examining higher-order cognitive function, specifically in studies of drug action or toxicant exposure.

Furthermore, forward and backward approaches were equally effective during the performance condition, but PQ was higher when backward chaining was used during the learning component, regardless of chain structure. Very low doses of D-amphetamine improved learning under some conditions. Among other things, these observations suggest that training procedure and chain structure may be of greatest importance during the acquisition of a response chain. Further, if not controlled for, these variables could be confounds in investigations of drug or toxicant effects on learning using the IRA procedure. These results supplement those of Wright and Paule (2007) and Cohn et al. (1993) who also noted the importance of chain type in the IRA procedure. The current implementation of an IRA procedure addressed two important procedural manipulations that were previously unaddressed in the literature and offered a more suitable dependent measure for a mastery-based IRA procedure.

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